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Urine Light Chain Ratio Combined with Immunofixation Electrophoresis for Preliminary Screening of Eldly MM Patients who had Renal Injury as the First Symptom

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Abstract **Objective:** Recently, the incidence of MM (Multiple myeloma, MM) in the elderly has gradually increased. We explored the diagnosis efficiency of urine light chain ratio combined with immunofixation electrophoresis for preliminary screening elderly MM patients who had renal injury as the first symptom. **Methods:** A total of 981 elderly outpatients, newly diagnosed with renal injury, in our hospital from January 2017 to February 2019 were retrospectively reviewed. According to the results of immunofixation electrophoresis, they were divided into M-protein group and non-M-protein group. The laboratory test data of each group were collected. **Results:** Among the 981 enrolled cases, 84 cases were in the M-protein group, accounting for 8.6%. There was no statistically significant difference in Crea and eGFR between the M-protein group and the non-M-protein group. The KAP/LAM ratio was higher in the non-M group then that in the M-protein group with LAM type light chain expression, and was lower in the M-protein group with KAP type light chain expression (P<0.01). Compared with IgG, IgA and IgM groups, Crea and u-mALB/Crea were increased and eGFR was decreased in the simple light chain group (P<0.05). Significant differences in renal function indicators in kidney diseases caused by different causes in 897 non-M protein groups. However, there was no statistically significant difference between Kappa/Lambda ratios in blood and in urine. **Conclusion:** Urine light chain ratio had a higher specificity for initial screening of elderly MM patients with renal injury as the first symptom. It could reflect the degree of monoclonal proliferation. Moreover, it was easy to be accepted by patients and suitable for health checkup or preliminary screening of suspected MM patients.

Keywords: *multiple myeloma, urine light chain ratio, immunofixation electrophoresis, early preliminary screening*

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1. Introduction

MM (Multiple myeloma, MM) is a malignant disease of the blood system with abnormal proliferation of monoclonal plasma cells. The incidence of MM in the elderly has gradually increased in recent decades [1]. Because elderly patients often suffer from a variety of diseases, most of them are diagnosed at advanced stages because of the lack of early manifestations and occult onset. Owing to its non-specific first symptoms, such as swelling, fatigue, dizziness and back pain, MM is commonly misdiagnosed as chronic nephritis, nephrotic syndrome and renal failure. Suzuki K reported that the first symptom of MM in about 50% of cases was proteinuria or renal failure [2]. The treatment for MM-induced renal damage is differently distinguished from other primary and secondary renal disease. Therefore, choosing appropriate laboratory testing indicators is of great significant for early clinical diagnosis of renal damage, which was caused by MM. A total of 981 elderly patients with newly kidney injury in our hospital from January 2017 to February 2019 were involved in this study. Retrospective analysis of relevant laboratory test data was conducted to explore the use of urine light chain ratio combined with immunofixation electrophoresis in the initial screening of elderly MM patients with renal injury as the first symptom.

2 Materials and Methods

2.1. Data Collection

From January 2017 to February 2019, clinical data about 981 elderly patients (age: 70.4±7.5 years old; male%: 61.16%) with newly diagnosed with renal injury in the outpatient department in our hospital were collected. The elderly patients were defined by age more than 60 years old and patients with MM history were excluded.

2.2. Experimental Methods

We collected fasting venous blood from above patients, and centrifuged the venous blood at 3000 rpm for 10 min to obtain serum. The patient's clean mid-stream urine was collected and centrifuged at 3000 rpm for 5 min, and its supernatant was collected for further detection.

The light chains of KAP and LAM in urine and serum were determined by nephelometry with BN-II Siemens automatic immunoassay. Besides, M-protein in serum was detected by automatic electrophoresis apparatus (HeLena, USA), and M-protein was classified by 5 antiserology (IgG, IgA, IgM, light chain KAP and light chain LAM). Serum creatinine (Crea) was measured by BeckmanAU5800 automatic biochemical analyzer, and the glomerular filtration rate (eGFR) was estimated by ckd-epi formula based on patient gender, age and Crea concentration. The ratio of urinary microalbumin to creatinine (u-malb /Crea) was determined by DCA2000 microalbumin analyzer (Siemens,).

2.3. Statistical Methods

SPSS22.0 was used for statistical analysis. Shapiro-Wilks test was used to test the normal distribution of the data, and the abnormal distribution data were presented as median and interquartile range. Mann-Whitney test was used to compare the measurement data between two groups. Non-parametric one-way analysis of variance (Kruskal-Wallis H test) was used to the comparison among multiple groups. Nemenyi test was used for the comparison between paired samples. P<0.05 was considered statistically significant.

3. Results

3.1. Clinical Data Analysis of 981 Patients with Renal Injury

A total of 981 elderly outpatients, newly diagnosed with renal injury, in our hospital from January 2017 to February 2019 were involved in our study. The main disease included mainly chronic renal failure mainly, nephrotic syndrome and diabetic nephropathy, which accounted for 70.1%. All patients had different degrees of renal injury (Table 1).

3.2. The Analysis of Serum Immunofixation Electrophoresis Results

Serum M-protein were detected and classified by immunofixation electrophoresis. There were 84 patients detected out M-protein, with a percentage of 8.6% (84/981) in the whole population. Further analysis of the type of M-protein found that IgG type accounted for 41.7% (35/84), IgA type accounted for 21.4% (18/84), IgM type accounted for 10.7% (9/84), and pure light chain accounted for 26.2% (22/ 84). In the M-protein group, LAM-type light chain was highly expressed (57/84), and the specific distribution was as follows: IgG group, 71% (25/35); IgA group, 44.4% (8/18); IgM group, 55.6 % (5/9); pure light chain group, 86.4% (19/22). The expression of immunofixation electrophoresis in the non-M-protein group showed a normal distribution pattern. The immunoelectropherogram of each group of patients is shown in Figure 1.

Table 1. Clinical data of 981 patients with renal injury

Information	Disease/case (%)	Reference range
Chronic renal failure (uremia)	471/981(48.0)	
Nephrotic syndrome	125/981(12.7)	
Diabetic nephropathy	92/981(9.3)	
Primary glomerulonephritis	28/981(2.9)	
Lupus nephritis	26/981(2.6)	
Others *	239/981(24.4)	
Crea/(μmol/L), median(P25-P75) #	161.70(90.35, 357.05)	44.0-133.0
eGFR/[mL/(min·1.73m2)], median(P25-P75) #	32.90(12.28, 66.66)	>60
KAP/(g/L), median(P25-P75)	2.76(2.04, 3.62)	1.70-3.70
LAM/(g/L), median(P25-P75)	1.61(1.25, 2.05)	0.90-2.10
KAP/LAM, median(P25-P75)	1.73(1.51, 1.97)	1.35-2.65
u-mALB/Crea/(mg/g), median(P25-P75) #	254.50(92.80, 443.10)	0.0-30.0
u-KAP/(g/L), median(P25-P75)	48.95(19.98, 104.25)	0-7.1
u-LAM/(g/L), median(P25-P75)	28.85(9.93, 72.55)	0-3.9
u-KAP/LAM, median(P25-P75)	1.80(1.44, 2.16)	0.75-4.50

* Others diseases included ANCA- related vasculitis, IgA nephropathy, IgG4-related nephropathy, hypertensive nephropathy.
#Crea, eGFR, u-mALB compared with normal range, $P<0.05$.

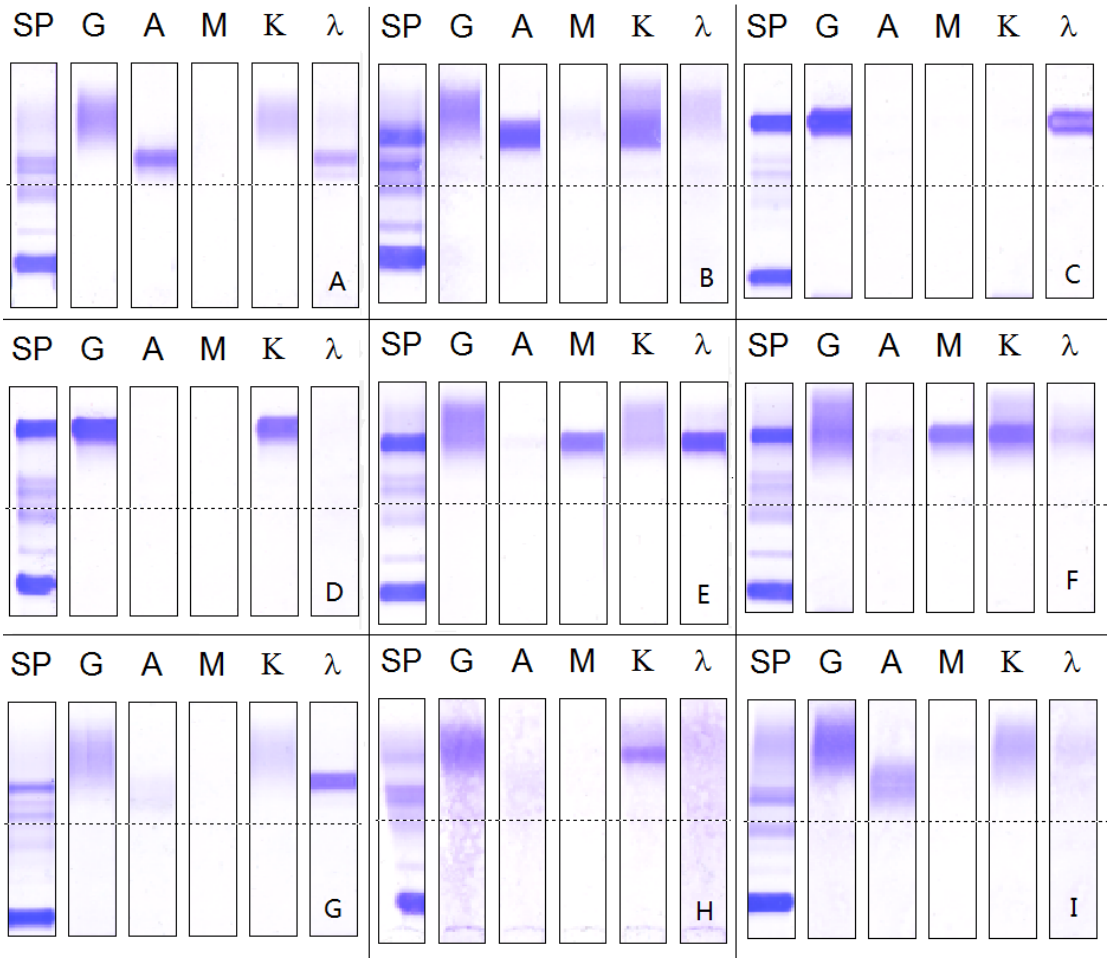

Figure 1. Immunofixation electrophoresis results (A: IgA-λ; B: IgA-κ; C: IgG-λ; D: IgG-κ; E: IgM-λ; F: IgM-κ; G: LAM light chain; H: KAP light chain; I: negative)

3.3. Analysis of Renal Function in Patients with M-protein and non-M-protein

There were no significant differences in Crea and eGFR between the M-protein group and the non-M-protein group. The KAP/LAM ratio of the M-protein group with KAP-type light chain expression was higher than that of the non-M-protein group. The KAP/LAM ratio of the M-protein group with LAM-type light chain expression was lower than that of the non-M-protein group (P<0.01, Table 2).

3.4. Analysis of Renal Function between Different M-protein Types

There were no significant differences in Crea, eGFR and u-mALB/Crea between the IgG group, the IgA group and the IgM group (Table 3). Compared with the IgG, IgA and IgM groups, the Crea and the ratio of u-mALB/Crea were increased in the pure light chain group, while the eGFR was decreased (P<0.05, Table 4).

Table 2. Comparison of renal function and blood and urine KAP/LAM ratios in patients with non-M-protein and M-protein groups [median (interquartile range)]

Item	Non-M-protein group(n=897)	Mproteingroup(n=84)	*P*
Crea/(μmol/L)	161.00(89.80, 345.85)	172.60(113.65, 412.58)	0.163
eGFR/[mL/(min·1.73m2)]	32.96(12.72, 67.65)	31.49(10.15, 55.05)	0.193
u-mALB/Crea/(mg/g)	250.30(99.30, 446.45)	307.00(72.75, 408.85)	0.802
KAP/LAM	1.74(1.55, 1.97)	KAP type: 4.12(1.90, 5.65)	0.000
		LAM type: 0.79(0.37, 1.13)	0.000
u-KAP/LAM	1.80(1.47, 2.15)	KAP type: 4.05(2.74, 7.37)	0.000
		LAM type: 0.68(0.03, 1.16)	0.000

Table 3. Analysis of renal function indicators among different heavy chain type groups in the MM group [median (interquartile range)]

Renal function	IgG group(n=35)	IgA group(n=18)	IgM group(n=9)	*P*
Crea/(μmol/L)	181.60(105.80, 396.70)	140.45(93.20, 359.10)	169.75(145.03, 393.45)	0.720
eGFR/[mL/(min·1.73m2)]	30.06(12.02, 58.70)	43.11(13.83, 57.41)	34.46(22.67, 41.96)	0,720
u-mALB/Crea/(mg/g)	346.30(148.60, 385.30)	53.40(28.80, 180.45)	201.40(6.60, 395.80)	0.115

Table 4. Analysis of renal function indexes between the light chain group and the heavy chain group in the MM group [median (interquartile range)]

Renal function	IgG/M/A group(n=62)	Light chain group(n=22)	*P*
Crea/(μmol/L)	151.90(105.80, 396.70)	289.70(117.45, 613.50)	0.043
eGFR/[mL/(min·1.73m2)]	40.00(12.40, 55.46)	12.70(6.11, 46.81)	0.016
u-mALB/Crea/(mg/g)	302.30(52.93, 387.15)	421.90(98.55, 588.85)	0.011

Table 5. Analysis of various indicators between different renal diseases in non-M-protein group

Item	Chronic renal failure (n=432)	Nephrotic syndrome (n=113)	Diabetic nephropathy (n=88)	Primary glomerulonephritis (n=25)	Lupus nephritis (n=26)	Others (n=213)	*P*
Crea (μmol/L)	223.50(133.78, 436.28)	88.40(69.95, 135.13)	128.70(93.80, 224.80)	71.80(61.10, 93.98)	190.30(107.33, 362.48)	141.15(78.15, 367.53)	0.000
eGFR [mL/(min·1.73m2)]	22.36(9.30, 43.75)	71.26(39.87, 89.56)	45.06(22.68, 68.36)	89.33(63.72, 94.63)	25.30(10.18, 42.97)	38.99(11.38, 78.50)	0.000
u-mALB/Crea (mg/g)	292.00(107.40, 485.85)	293.80(184.60, 420.20)	375.55(173.13, 514.53)	16.70(9.50, 168.50)	330.40(169.53, 429.10)	148.80(52.30, 355.00)	0.000
KAP/LAM	1.77(1.59, 2.01)	1.73(1.58, 2.08)	1.74(1.61, 1.93)	1.76(1.44, 1.88)	1.79(1.63, 2.01)	1.74(1.54, 1.97)	0.886
u-KAP/LAM	1.81(1.49, 2.15)	1.79(1.57, 2.16)	1.84(1.57, 2.32)	1.86(1.81, 2.77)	1.58(1.46, 1.86)	1.81(1.54, 2.28)	0.285

3.5. Analysis of Various Indicators among Different Renal Diseases in non-M-protein Group

897 patients with non-M-protein group were divided into different groups according to the cause of renal injury. The results of each index are shown in Table 5. Renal function indicators (Crea, eGFR, u-mALB/Crea) were significantly different in renal diseases caused by different causes. There was no statistically significant difference in blood and urine KAP/LAM ratios, and the difference between the groups was not statistically significant when compared with the normal range.

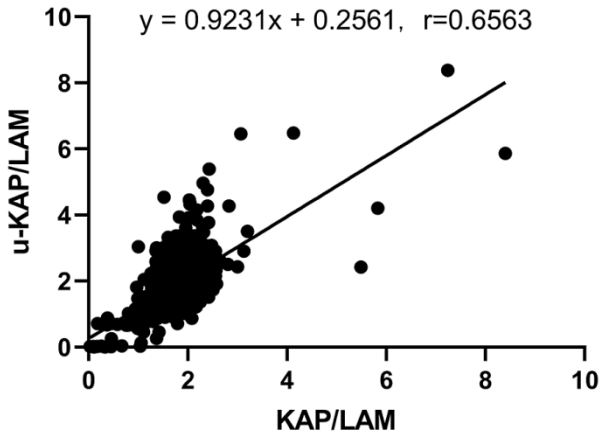

Figure 2. The correlation between blood and urine KAP/LAM ratio

3.6. Comparison and Correlation Analysis of the Ratio of Blood to Urine KAP/LAM in the Diagnosis of M Protein

The results of serum immunofixation electrophoresis and the final results of bone marrow cytology were used as the diagnosis criteria. The sensitivity of urinary KAP/LAM ratio diagnosis of M-protein (55.5%) was lower than that of blood KAP/LAM ratio (78.4%), but its specificity (93.6 %) was higher than the blood KAP/LAM ratio (91.3%). There was a good correlation between blood and urine KAP/LAM ratio (P<0.01, r=0.6563, Figure 2).

4. Discussion

MM is characterized by anemia, abnormal increase of plasma monoclonal immunoglobulin and/or light chain fragments, abnormal light chain protein (Bence-Jones protein) in urine, osteolytic damage and renal dysfunction. These lesions commonly accumulated in multiple organs. Renal insufficiency is one of the usual clinical manifestations [3]. Primary renal disease is completely different from MM renal damage treatment. Once the patient was diagnosed with MM, chemotherapy should be performed in time to reduce the content of abnormal monoclonal immunoglobulin and light chain protein in serum, thereby reducing renal damage. Therefore, how to diagnose MM-induced renal disease as soon as possible and distinguish it with primary renal disease played the important clinical value.

The current gold diagnosis standard for MM and primary renal disease are bone marrow cytology and renal biopsy. However, both of them were invasive. Moreover, when the indications are not sufficient or the patient has bone marrow or contraindication of renal biopsy, a non-invasive method is needed for the assisted diagnosis [4,5,6,7]. The main laboratory feature of MM is that monoclonal proliferating plasma cells produce a large amount of M-protein. And, immunofixation electrophoresis is the most commonly used method for detecting M-protein in clinical practice. However, this method has limitations of long detection period, requiring special instruments and professionals to interpret the results and the cost of testing is expensive. It is not suitable for early screening and detection and difficult to carry out in primary hospitals.

Normal plasma cells produce two light chains, KAP and LAM, which are assembled with different heavy chains into complete immunoglobulin. In the MM patients, the proliferation of malignant monoclonal proliferation of plasma cells produced a monoclonal immunoglobulin light chain, while inhibiting the formation of another light chain, so the serum light chain ratio of MM patients would be significantly abnormal [8,9]. The content of serum light chain is usually a reflection of the degree of monoclonal proliferation of plasma cells in the bone marrow. The greater the difference in ratio, the more severe the

disease. In addition, the imbalance of the ratio is an important indicator for distinguishing MM from other diseases. Momeni A et al. reported that serum light chain ratio determination has important value in the diagnostic screening, the efficient evaluation and prognosis of MM [10]. The urine light chain ratio can also reflect the degree of monoclonal proliferation, and it is easily accepted by patients because the sample can be collected more convenient and non-invasive. Owing to the highly automated measurement, the results are easy to interpret and the cost is relatively low. Therefore, this study aimed to explore its value in the diagnosis of MM-induced renal insufficiency in elderly patients.

In the 981 patients newly diagnosed with renal damage, 84 patients (8.6%) were found to have M-protein in the serum by immunofixation electrophoresis. These patients were finally diagnosed as MM by bone marrow cytology. Meanwhile, they were diagnosed with proteinuria and chronic renal insufficiency at the time of initial diagnosis. MM were not screened in the primary and secondary renal injury patients caused by diseases such as diabetic nephropathy, hypertensive nephropathy, lupus nephropathy, and chronic glomerulonephritis. Therefore, the results suggested that for elderly patients with unexplained renal insufficiency, especially after excluding diabetes, hypertension, and autoimmune diseases, MM may be considered. It is recommended that the urine KAP/LAM ratio might be detected first. Immunofixation electrophoresis and serum KAP, LAM test could be used to exclude MM, reduce misdiagnosis and missed diagnosis.

The results of this study showed no significant difference in Crea, eGFR and mALB/Crea between M-protein group and non-M-protein group. However, Crea and mAb/Crea showed an increasing trend in the M-protein group, while eGFR showed a decreasing trend. It was suggested that the renal function damage may be more serious in the M-protein group. There was a significant difference in blood and urine KAP/LAM ratio between the two groups. The ratio of patients with KAP-type light chain expression was significantly higher than that of non-M white patients, while the ratio of patients with LAM-type light chain expression was significantly lower than that of non-M-protein patient group. We found that light chain ratio determination may play an important role in MM with renal insufficiency and was worthy of attention. At the same time, there was no significant difference in light chain ratio between patients with various types of renal injury in non-M-protein group and they were all within the normal reference range of KAP/LAM ratio. KAP/LAM ratio could be used to differentially diagnose MM-induced renal damage and it was of great significant in simple renal injury. Comparing blood and urine KAP/LAM ratios in MM diagnostic value, urine KAP/LAM ratio has the higher specificity. The sensitivity is slightly lower than the serum ratio, which may be attribute to many LAM type-cases. The LAM-type light chain often forms a dimeric structure, and the increase in relative molecular mass slowed its clearance in the renal, making the LAM-type light chain more likely to accumulate in the renal [11] and resulting in urine LAM during the test. The high concentration of the light chain produced a "hook effect", which caused the inaccurate detection result of the urine KAP/LAM ratio.

In the study, we also found that patients with M-protein group were mainly expressed by LAM light chain (67.9%). Meanwhile, we found that patients with pure light chain group had significantly higher serum Crea level and lower eGFR level than other types of MM-protein patients. These results suggested that MM patients expressing LAM light chain were more prone to renal damage, and the level of renal damage was more serious, which was the same as that of domestic and foreign scholars [12,13,14]. This may be explained by the pathological mechanism of MM. MM-induced renal damage was mainly caused by the tubular nephropathy and the abnormal proliferation of immunoglobulin in the renal tubule. A large number of light chains accumulate in the renal tubules, block the renal tubules, and eventually leaded to chronic renal failure. Additionally, the light chain could cause amyloidosis of the glomerulus, stimulate the production of cytokines, cause chronic inflammation and fibrosis.

The highlight of this study is the discovery of an economical and rapid MM diagnostic screening method. This study demonstrated that the urinary KAP/LAM ratio was useful for diagnosing elderly MM patients, and the sample retention was convenient, non-invasive, rapid, and inexpensive. It's ideal for health-checkup or early patient screening. However, there are some shortcomings. Firstly, this study was a single-center study with limited coverage. The multi-center analysis would be conducted to verify the conclusion in the future. Secondly, the detection of free light chain in serum and urine was not performed. And the classification of free light chains in immunofixation electrophoresis may lead to the bias in the final results.

5. Conclusion

It is recommended elderly patients with renal injury to perform urine light chain ratio test during routine urine examination. Based on the ratio results, it could be initially judged whether it is the simple renal insufficiency or renal damage caused by MM. The detection of serum immunofixation electrophoresis and light chain ratio for patients with abnormal ratios might be meaningful in judging the nature of the disease. Thereby, it could improve the early diagnosis rate of MM and gain valuable insights for active treatment and prognosis.

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